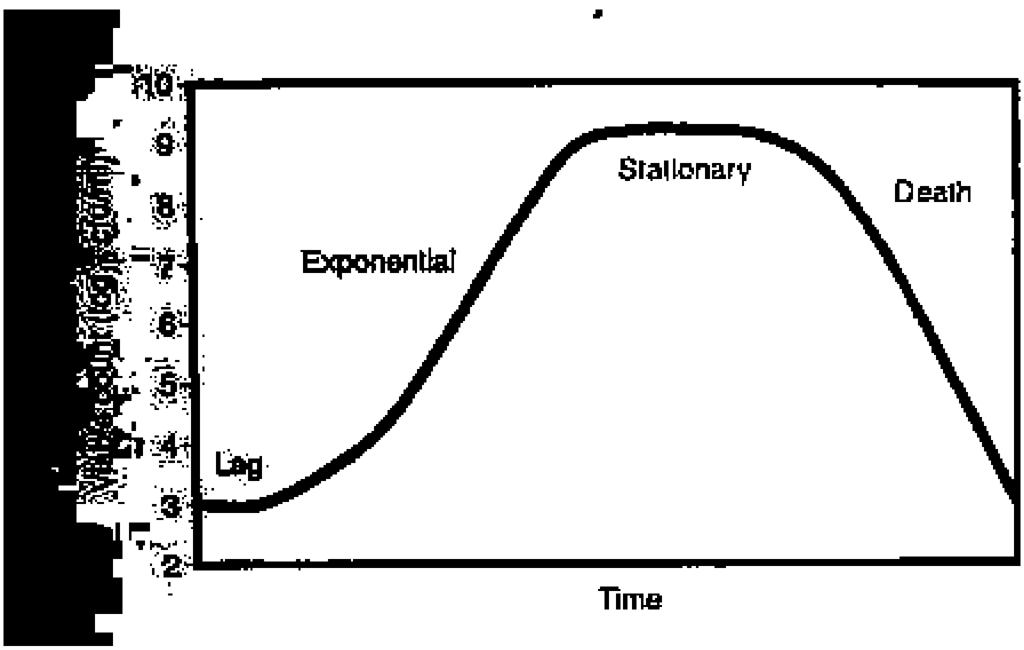
Food Microbiology Lecture No. 1
Basic Microbiology

- Microorganisms: protozoa, fungi, bacteria, viruses and prions (unculturable infectious diseases)
- Prions: a protein particle that lacks nucleic acid and is believed to be the cause of various infectious diseases of the nervous system (as bovine spongiform encephalopathy and Creutzfeldt-Jakob disease)
- Eucaryotic or Eukaryotic: mitochondria, endoplasmic reticulum, and defined nucleus: yeast and mold (mould)
- Procaryotic or Prokayotic: no obvious organelles, same size of EK organelles: bacteria
- Evolution of life from PK to EU: *intracellular symbiotic relationship*
- Importance of m/o in foods
- Intestinal parasites: the pork tapeworm (*Taenia solium*) beef tapeworm (*T. saginata*) freshwater fish (*Diphyllobothrum latum*)
- Contaminated water: pathogenic protozoa
- Yeasts: wine and beer/ spoilage in sugar products
- Molds: Cheeses (blue cheese)/ mycotoxins
- Bacteria cell structure
  - Unicellular m/o
  - Bacilli (*Bacillus* sp), cocci (*Streptococcus* and *Staphylococcus*), spiral (*Vibrio* and *Campylobacter*)
  - Gram stain: Christain Gram (Danish microbiologist)
  - Dark blue or red
  - Precipitation of crystal violet with iodine in the cell cytoplasm could not be extracted by using ethanol from Gram positive (blue)
  - Ethanol extracts the crystal violet-iodine complex from Gram negative m/o (thinner peptidoglycan layer or murein and no teichoic acids): counter stain using safranine or basic fuschin
  - Gram positive m/o have thick peptidoglycan layer or murein and no teichoic acids
  - Peptidoglycan is a site of action for Penicillin G (original *Penicillin*). This explains why it only is effective against streptococci and staphylococci (gram positive)
  - Lipopolysaccharide (LPS, O antigen): the outer membrane of gram-negative m/o contains LPS
  - LPS: lipid A, core and O antigen
  - Lipid A: Virulent factor in *Salmonella*
  - Fragella (H antigen)
  - Most rod-shaped bacteria are motile in liquid media
  - Single flagellum (monotrichus)
  - Tuft of fragella (lophotrichus) one or both poles
  - Many fragella (peritrichus)
  - H (german: hauch meaning breath) Proteus swarming on moist agar plate: similar to the light mist cause by breathing on cold glass

- Fragella is protein (antigen), denature by heating (100C for 20 min), acid and alcohol
- E. coli has 173 O-antigens and 56 H-antigens
- E. coli O157:H7
- <u>Capsule</u> (*Vi* antigen)
- Slimy polymeric material (polysaccharides) if the layer is very dense: called capsule
- Resistant to white blood cell engulfment
- Thought to be virulent of *Salmenella typhi*

<u>Microbial growth cycle</u>

- Lag phase
- Acceleration phase
- Log phase (exponential phase)
- Deceleration phase
- Stationary phase
- Death phase



Food Microbiology Lecture 2
<u>Basic Microbiology Continued</u>

- Lag phase: cells are not multiplying but synthesizing enzymes
- Acceleration phase: Multiplying
- Log phase: Double in number
- Deceleration phase: no longer multiply
- Stationary phase: Rate of growth = rate of death
- Death phase: cells start to die

Microbial Death

- D-Value: decimal reduction time. Time at any given temperature required to kill 90% of population
- Z-value: temperature increase required to reduce the D-value 10 fold

Factors affecting microbial growth

- Intrinsic factors
  - Water activity; Bacteria> Yeast> Mold
  - Oxygen availability
  - pH: Low acid foods, acid foods (4.5 and lower)
  - Buffer capacity: change of pH
  - Nutrients
  - Natural antimicrobial substances
  - Microflora
- Extrinsic factors
  - Temperature: Psychrophiles 12-15C/ Mesophiles 30-54C/ Thermophiles 55-75C
  - Relative humidity
  - Atmospheric condition: Aerobic/ Anaerobic/ Facultative anaerobic / Microaerophilic
    - Obligated aerobic➔ mold
    - Obligated anaerobic➔ *Clostridium botunilum*
    - Facultative anaerobic ➔ *E. coli*
    - Microaerophilic➔ *Campylobacter jejuni*

Food Microbiology Lecture 3

Factors affecting microorganisms

Effect of temperature: affects cell membrane and enzymes

- *E. coli* min-8C, optimum 37C, Max 47C
- Optimum of *Clostridium perfringens* 37-45C
- Terms:

Table 6.1 Groups of micro-organisms based on growth temperatures

Group	Minimum °C	Optimum °C	Maximum °C
Obligate psychrophile	−10	10–15	20
Psychrotroph	−10	20–30	42
Mesophile	5	28–43	52
Thermophile	30	50–65	70
Extreme thermophile	65	80–90	100

Table 6.2 Cardinal temperatures for a range of micro-organisms

Organism	Min °C	Opt °C	Max °C	Group
Flavobacterium	−10	10	20	Obligate psychrophile
Pseudomonas fragii	−6.5	24	38	Psychrotroph
Escherichia coli	8	37	45	Mesophile
Bacillus stearothermophilus	28	55	72	Thermophile
Bacillus subtilis	10	28	51	Mesophile
Bacillus coagulans	30	45	56	Mesophile/thermophile?
Pseudomonas fluorescens	−4	26	40	Psychrotroph
Thermococcus spp	65	85	95	Extreme thermophile
Aeromonas spp	0	28	42	Psychrotroph
Arthrobacter glacialis	−5	15	20	Obligate psychrophile
Desulfotomaculum nigrificans	30	55	71	Thermophile

- Mesophilic food poisoning: *Salmonella, Staphylococcus, Clostridium, Campylobacter jejuni, Vibrio parahaemolyticus, Bacillus cereus*
- Associated with Chilled foods: *Bacteria: Pseudomonas, Alterromonas, Shewanella, Bacillus, Clostridium, Lactobacillus,/Yeast: Candida, Torulopsis, Saccharomyces,/ Mold: Penicillium, Aspergirus, Alternaria*

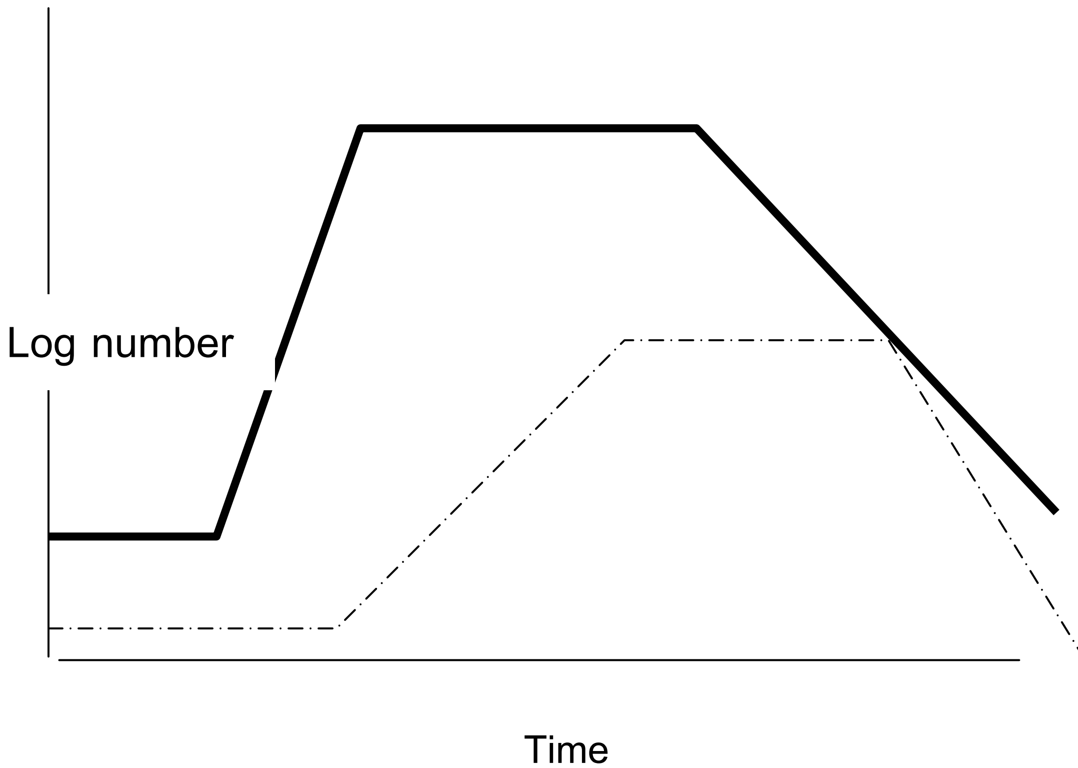
Food Microbiology Lecture 4
Factors affecting microorganism

Effect of Freezing on M/O:

- Liquid water ➔ Ice crystal, lower Aw
- Most frozen foods: -0.5 to -3.0 C
- Very few bacteria can grow below -5C
- Yeast, *Debaromyces* grows at -12.5C
- Gradually die
- G- is more sensitive to freezing than G+
- Bacteria spores and viruses are unaffected
- Stationary phase cells are more resistant (less liquid in cell membrane)
- Very low temperature can preserve cells
- Acid food increases freezing damage
- Treatments before freezing
- Time of storage
- Rate of thawing: Fast freezing ➔ slow thawing, ice crystal increases in size
- But Fast freezing ➔ fast thawing does not produce this effect
- Slow freezing, ice forms outside the cells. Conc of solutes increases➔ plasmolysis, cell shrinkage,and most lethal
- Fast freezing, ice forms inside the cells. Internal cell solutes increase, pH changes ➔ damage cell proteins. Also mechanical damage by ice crystal.
- Freezing injury of cells: Not killed and can be recovered. Damaged cells may need some special nutrients. Normally injured cells are readily recovered in thawed foods.

Water activity

- Living organisms have 75% water
- Dormant forms (spores-15%) have much less water
- Unavailable water
    - Water contains dissolved solutes (sugar)
    - Crystallized as ice
    - Water is absorbed onto surfaces (matrix effect)
- Minimum Aw required by
    - G- 095
    - G+ 0.90
    - Yeast 0.85
    - Mold 0.80
    - Extreme halophile 0.71 (salt)
    - Xerophillic mold 0.61 (dry)
    - Osmophillic yeast 0.62 (osmotic pressure)
- When lower Aw: slower growth rate, increase the length of lag phase, fewer cells in stationary phase and die more rapidly



- Cells in Low Aw (in salt or sugar solutions)= hypertonic environment (more concentrated than dissolved materials in the cell cytoplasm), water flow out of the cells
- Osmotic damage and cell death
- Damage to cell membrane, damage to enzymes
- Isotonic, hypotonic and hypertonic environments

Effect of pH on microbial growth

$$H_2O \leftarrow\rightarrow OH^- + H^+$$
$$[H^+] = [OH^-] = 1x\ 10^{-7}\ mol/L$$

If a solution has $[H^+] > [OH^-]$ = acid
If a solution has $[H^+] < [OH^-]$ = base
If a solution has $[H^+] = [OH^-]$ = neutral

pH is a measure of $[H^+]$

A solution containing 10^{-7} mol/L → pH 7
A solution containing 10^{-5} mol/L → pH 5
A solution containing 10^{-8} mol/L → pH 8

pH 4 is 1000 time more acidic than pH 7
Effects:

- Growth rate decreases

- Number of cells drops
- Longer lag phase
- Shorter stationary phase
- Death rate increases

Internal pH of bacteria = 7, yeast =5.8

- Moderate pH change affects cell membrane and enzymes, internal pH is not affected
- Extreme pH change affects internal pH, Protein denature, cell dies

Effect of weak acid on microbial growth is temperature depent. High temp increases its effects

Antimicrobial effect of acids

Propionic > acetic > lactic > citric > phosphoric > hydrochloric

pH of culture media: pH drift:

- Sugar metabolized by fermentative m/o, acidic
- Amino acid is used ➔ ammonia ➔ alkaline
- Ammonia chloride is used ➔ acidic

AA in media is Buffer/ add acid, base solution

Food Microbiology Lecture 5

<u>Food Preservatives</u>

- To extend the shelf-life of Food while maintain its wholesomeness and freshness as much as possible
- Many preservatives are effective under low pH condition:
    - Benzoic acid pH<4
    - Propionic acid pH<5
    - Sorbic acid pH<6.5
    - Sulfites pH<4.5
- Parabens (benzoic acid esters) are more effective at neutral pH conditions

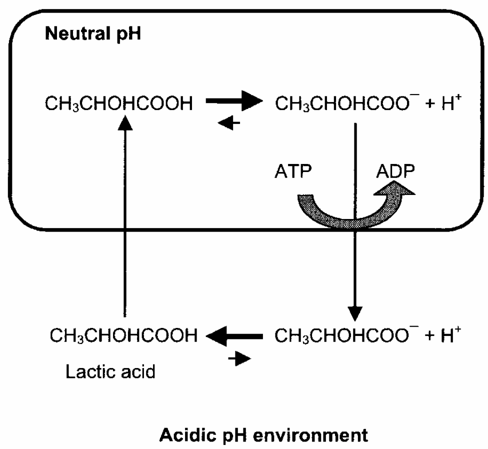
Table 2.12 Antimicrobial food preservatives (adapted from Gould and reprinted with permission of Elsevier Science from the *International Journal of Food Microbiology*, 1996, **33**, 51-64).

Preservative (typical concentration range, mg/kg)	Examples of use
Weak organic and ester preservatives	
Propionate (1-5000)	Bread, bakery and cheese products
Sorbate (1-2000)	Fresh and processed cheese, dairy products, bakery products, syrups, jams, jellies, soft drinks, margarines, cakes, dressings
Benzoate (1-3000)	Pickles, soft drinks, dressings, semi-preserved fish, jams, margarines
Benzoate esters (parabens, 10)	Marinaded fish products
Organic acid acidulants	
Lactic, citric, malic, acetic acids (no limit)	Low pH sauces, mayonnaises, dressings, salad creams, drinks, fruit juices and concentrates, meat and vegetable products
Inorganic acid preservatives	
Sulphite (1-450)	Fruit pieces, dried fruit, wine, meat sausages
Nitrate and nitrite (50)	Cured meat products
Mineral acid acidulants	
Phosphoric acid, hydrochloric acid	Drinks
Antibiotics	
Nisin	Cheese, canned foods
Natamycin (pimaricin)	Soft fruit
Smoke	Meat and fish

Organic acids

- Acetic, lactic, benzoic, and sorbic
- Inhibits Bacteria and fungal cells
- Sorbic acid inhibits bacteria spore germination
- Addition of 0.2% calcium propionate to bread dough delays *B. cereus* germination (Kaur, 1986)
- Use bezoic acid at 500 ppm (mg/L) to preserve fruit juice-based beverages

- Sulphur dioxide used in many foods is limited to 10 ppm by European regulations
- Weak acid = pH dependent
- Low pH flavors discharged or undissociated state of acid molecule
- It can be freely permeable across plasma membrane (lipophillic) and thus able to enter cells
- Once entered the cells (higer pH), it will dissociate and release charged anions and protons which can not across the membrane.
- Result: accumulation of anions and protons inside the cell
- Inhibits metabolisms, change of internal pH, cell membrane disruption, accumulation of toxic anions



- In a yeast study, Yeast tried to restore homeostasis and results in the reduction of available energy pool
- To expel the charge anions out of cells, yeast uses ATP

Table 2.13 Inhibitory concentration (%) of dissociated organic acids (Various sources including Mortimore and Wallace, 1994; Borch *et al.*, 1996 and ICMSF, 1996).

Acid	Enterobacteriaceae	Bacillaceae	Yeasts	Moulds
Acetic	0.05	0.1	0.5	0.1
Benzoic	0.01	0.02	0.05	0.1
Sorbic	0.01	0.02	0.02	0.04
Propionic	0.05	0.1	0.2	0.05

Lactoperoxidase System

- Found in milk, against fungi and bacteria
- Requires hydrogen peroxide and thiocyanate
- Active agents: singlet oxygen, hydroxyl radical and superoxide radical (extremely biocidal)
- G- are more sensitive
- Extremely sporicidal at high temperature
- H_2O_2 is used in cheese, raw milk

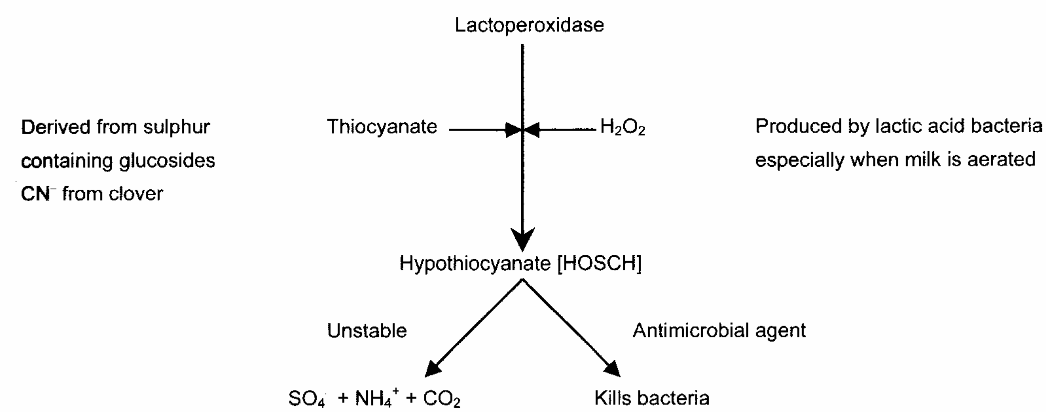


Fig. 2.8 The lactoperoxidase system.

Chelators:

- Such as citric acid, disodium and calcium salts of ethylene diaminetetraacetic acid (EDTA)
- EDTA, against G-
- Citric acid inhibits the growth of proteolytic *Cl. botulinum* due to Ca^{2+} chelating activity

Small organic biomolecules

- Naturally present in spices
- Eugenol in cloves (กานพลู) and cinnamon (อบเชย)
- Allicin in garlic (กระเทียม)
- Thymol in rosemary
- Thiocyanate in mustard
- Hydrophobic
- Membrane rupturing characteristics

- Debating: fresh garlic/Cooked garlic
- Essential oil from plants such as basil, cumin, caraway (คล้ายยี่หร่า) and coriander (คล้ายผักชี)

Table 2.14 Concentration of essential oils in some spices and antimicrobial activity of active components (adapted from ICMSF 1998).

Spice	Essential oil in whole spice (%)	Antimicrobial compounds in distillate or extract	Antimicrobial concentration (ppm)	Organisms
Allspice	3.0–5.0	Eugenol	1000	Yeast
(*Piementa dioica*)		Methyl eugenol	150	*Acetobacter*
				Cl. botulinum 67B
Cassis	1.2	Cinnamic aldehyde	10–100	Yeast
(*Cinnamomum cassis*)		Cinnamyl acetate		*Acetobacter*
Clove	16.0–19.0	Eugenol	1000	Yeast
(*Syzgium aromaticum*)		Eugenol acetate	150	*Cl. botulinum*
				V. parahaemolyticus
Cinnamon Bark	0.5–1.0	Cinnamic aldehyde	10–1000	Yeast, *Acetobacter*
(*Cinnamomum zeylanicum*)				*Cl. botulinum* 67B
		Eugenol	100	*L. monocytogenes*
Garlic	0.3–0.5	Allyl sulphonyl	10–100	*Cl. botulinum* 67B
(*Allium sativum*)		Allyl sulphide		*L. monocytogenes*
				Yeast, bacteria
Mustard	0.5–1.0	Allyl isothionate	22–100	Yeast, *Acetobacter*
(*Sinapis nigra*)				*L. monocytogenes*
Oregano	0.2–0.8	Thymol	100	*V. parahaemolyticus*
(*Origanum vulgare*)		Carvacrol	100–200	*Cl. botulinum* A, B, E
Paprika		Capsicidin	100	*Bacillus*
(*Capsicum annuum*)				
Thyme	2.5	Thymol	100	*V. parahaemolyticus*
(*Thymus vulgaris*)		Carvacrol	100	*Cl. botulinum* 67B
				Gram-positive bacteria
				Asp. parasiticus
				Asp. flavus
				aflatoxin B_1 and G_1

Food Microbiology Lecture 6
<u>Microbiology Response to Stress</u>

- Inhibition, Injury, Inactivation
- Consumers demand minimally processed foods
- Less processed, less heavily preserved, higher quality foods
- Rely on storage and distribution at refrigeration temperatures
- Concerns: psychrotrophic and mesophic m/o

Table 2.15 Food poisoning microorganisms of concern in minimally processed foods (adapted from Abee & Wouters 1999).

Minimum growth temperature (°C)	Heat resistance	
	Low[a]	High[b]
0–5	*L. monocytogenes* *Y. enterocolitica* *A. hydrophila*	*Cl. botulinum* type E and non-proteolytic type B *B. cereus* *B. subtilis* *B. licheniformis*
5–10	*Salmonella* spp. *V. parahaemolyticus* Pathogenic strains of *E. coli* *St. aureus*	
10–15		*Cl. botulinum* type A and proteolytic type B *Cl. perfringens*

[a] Organisms undergo a 6 log kill following heat treatment at 70°C for 2 minutes.
[b] Organisms require heat treatment at 90°C or above to destroy spores.
Note: To convert to °F use the equation °F = (9/5)°C + 32. As a guidance: 0°C = 32°F, 4.4°C = 40°F, 60°C = 140°F.

- Adaptation: Gene expression
- Cold shock, Heat shock, Weak acids, High osmolarity, high hydrostatic pressure
- Example: *Bacillus and Clostridium*: produce spores under stress conditions
- Sigma factor binds to core RNA polymerase
- Sigma factor helps to produce promoters (to promote a gene to express)

Table 2.16 Response mechanisms in microorganisms (adapted from Gould and reprinted with permission from Elsevier Science from the *International Journal of Food Microbiology*, 1996, **33**, 51-64).

Environmental stress	Stress response reaction
Low nutrient levels	Nutrient scavenging, oligotrophy, generation of viable non-culturable forms
Low pH, presence of weak organic acids	Extrusion of hydrogen ions, maintenance of cytoplasmic pH and membrane pH gradient
Reduced water activity	Osmoregulation, avoidance of water loss, maintenance of membrane turgor
Low temperature - growth	Membrane lipid changes, cold shock response
High temperature - growth	Membrane lipid changes, heat shock response
High oxygen levels	Enzymic protection from oxygen-derived free radicals
Biocides and preservatives	Phenotypic adaptation and development of resistance
Ultraviolet radiation	Excision of thymine dimers and repair of DNA
Ionizing radiation	Repair of DNA single strand breaks
High temperature - survival	Low water content in the spore protoplast
High hydrostatic pressure - survival	Low spore protoplast water content?
High voltage electric discharge	Low conductivity of spore protoplast
Ultrasonication	Structural rigidity of cell wall
High levels of biocides	Impermeable outer layers of cells
Competition	Formation of biofilms, aggregates with some degree of symbiosis

Food Microbiology Lecture 6
Microbial flora of Foods

Food Spoilage

1. Insect damage
2. Physical injury
3. Activity of enzymes
4. Chemical changes
5. Activity of microorganisms

Spoilage Microorganisms

1. Gram negative spoilage m/o: *Pseudomonas, Alteromonas, Shewanella putrefaciens* and *Aeromonas* spp. Spoil dairy products, red meat, fish, poultry and eggs during cold storage. High Aw and neutral pH

*Heat stable protease and lipases→ off-flavor

2. Gram positive, non-sporeforming: LAB and *Brocothrix thermosphacta* spoil meat stored under modified atmosphere packaging. *Pediococcus* produce a thick polysaccharide slime in beer. LAB spoils wine (Lactic acid) giving sour taste

*Bacillus cereus*can grow in pasteurized milk at 5C ➔ sweet curdling (coagulation without acid) and bitty cream

Bacillus stearothermophilus ➔flat sour spoilage of canned foods (acid without gas)

*Desulfotomaculum nigrificans ➔ Sulphur stinker spoilage ➔Hydrogen sulphide (swell and smell)

3. Gram positive sporeforming spoil m/o

Bacillus and *Clostridium* ➔ heat-treated foods

4. Yeast and Mold

More tolerant of low Aw and low pH

Spoil fruit, vegetables and bakery products

*produce pectinoltic enzymes which soften the plant tissues causing rot.

- 30% of fruit spoilage ➔ *Penicillium*

Spoilage of Dairy Products:

- Ideal growth medium
- Intrinsic m/o = 100-10,000 cfu/ml from cow and equipment
- *Pseudomonas, Alcaligenes, Aeromonas, Acinetobacter, Moraxella, Flavobacterium, Microoccus, Streptococcus, Corynebacterium* and *Lactobacillus*
- Psychrotrophilic m/o ➔ heat stable lipase and proteases (not denatured during pasteurization)
- Lipase ➔ short-chain fatty acid➔ rancid
- Proteases ➔ bitter peptides
- Pasteurization kills pathogens such as *Mycobacterium tuberculosis, Salmonella* and *Brucella* spp.
- Thermoduric m/o survive ➔ Streptococcus thermophilus, *Enterococcus faecalis, Micrococcus luteus.*

Spoilage of Meat and Poultry Products

- Highly perishable, high Aw
- Protein ➔ high Buffer ➔ stable pH
- Sterile inside
- Become contaminated during slaughter, processing and storage
- *Pseudomonas, Brochothrix thermophacta* and LAB
- Pathogens: *Salmonella, E. coli, Listeria monocytogenes, Clostridium perfringen* and *Streptococcus aureus, Campylobacter jejuni*

Food Microbiology Lecture 7

Microbial flora of food

Spoilage of Fish

- Fish skin normally has $10^3 – 10^5$ cfu/cm^2
- Gills: $10^3 – 10^4$ cfu/g

- Intestine: $10^2 - 10^9$ cfu/g
- North seas: Psychrotrophs (Pseudomonas, Alteromonas, Shewanella, Acinetobacter
- Warmer water: mesotrophs: micrococci, coryneforms
- Can be rapidly spoilaged (7C for 5 day, 10^8)
- Faster than meat spoilage
- Off-odor: *Pseudomonas* sp. Produces volatile ester (ethyl acetate), volatile sulphide compound (methyl mercaptan, dimethyl sulphide)
- Spoiled fish odor: trimethylamine oxide is reduced by *Shewanella putrefaciens*

Egg spoilage

- Natural antimicrobial agents: iron chelating agents (conalbumin) and lysozyme in the albumen (egg white)
- Shell is covered with a water repellent cuticle and two inner membranes
- *Pseudomonas* sp., *Proteus vulgavis, Alteromonas spp., Serratia marcescens*

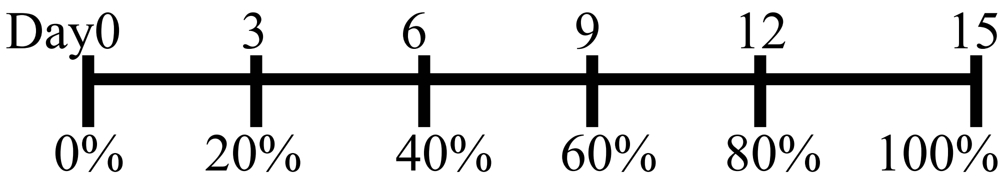
Shelf-Life Indicators

- Time between the production and the packaging of product and the point at which it becomes unacceptable to the consumer
- Depends on intrinsic and extrinsic factors

Table 4.3 Food products and associated shelf lives.

Food product	Typical shelf life
Bread	Up to 1 week at ambient temperature
Sauces, dressings	1 to 2 years at ambient
Pickles	2 to 3 years at ambient
Chilled foods	Up to 4 months at 0–8°C
Frozen foods	12 to 18 months in freezer cabinets
Canned foods	Unlacquered cans, 12 to 18 months Lacquered cans, 2 to 4 years

- Shelf-life determination
    1. Direct determination and monitoring: take sample at intervals equal to 20% of expected shelf-life to give samples of six different ages. Samples are stored under controlled conditions until their quality becomes unacceptable. Evaluated smell, texture, flavor, color and viscosity. Not ideal method for canned foods.



2. Accelerated estimation: Store at high temperature. However different m/o may require different temperature to growth.

Table 4.4 Suggested microbiological limits for end of shelf life.

Product	Microbial count	Comments
Raw meat	1×10^6 cfu/g aerobic plate count	Visible deterioration and/or slime at 1×10^7 cfu/g
Ground beef	1×10^7 cfu/g	End of shelf life, colour begins to fade and slime forms
Vacuum packaged cooked products	1×10^6 cfu/g aerobic plate count	Represents the point where a pH shift >0.25 occurs
Fully cooked products	1×10^6 cfu/g aerobic plate count 1×10^3 cfu/g Enterobacteriaceae 1×10^3 cfu/g lactic acid bacteria 500 cfu/g yeast and moulds	

Measuring compounds associated with microbial growth:

- Glucose: Main substrate for microbial growth of red meats (Modified and vacuum packaged). *Pseudomonas* uses glucose➔ measuring glucose depletion.
- Glucomic and 2-oxogluconic acid: *Pseudomonas* uses glucose resulting in the accumulation of Glucomic and 2-oxogluconic acids in beef.
- Lactic acids, acetic acid and ethanol: products of glucose metabolism of m/o. for example: acetate level greater than 8mg/100g meat indicate a microbial flora of $>10^8$ cfu/g

Microbiological Quality of Foods

- Storage trials: Store food samples and analyzed for total or specific microbial loads. – Total plate count or Pseudomonas. Lactic acid bacteria …Compare with sensory and other chemical/ physical properties of foods
- Challenge tests: Samples are inoculated with targeted m/o and incubated.
- Predictive modeling: Use a computer software to predict the growth of m/o under specific conditions and foods.

Food Microbiology Lecture 8

Methods of Food Preservations

1. Heat (Pasteurization, Sterilization)
2. Low temperature (Chilling, Freezing)
3. Dehydration
4. Fermentation
5. Chemical additive
6. Salting/Sugaring
7. Irradiation

Pasteurization

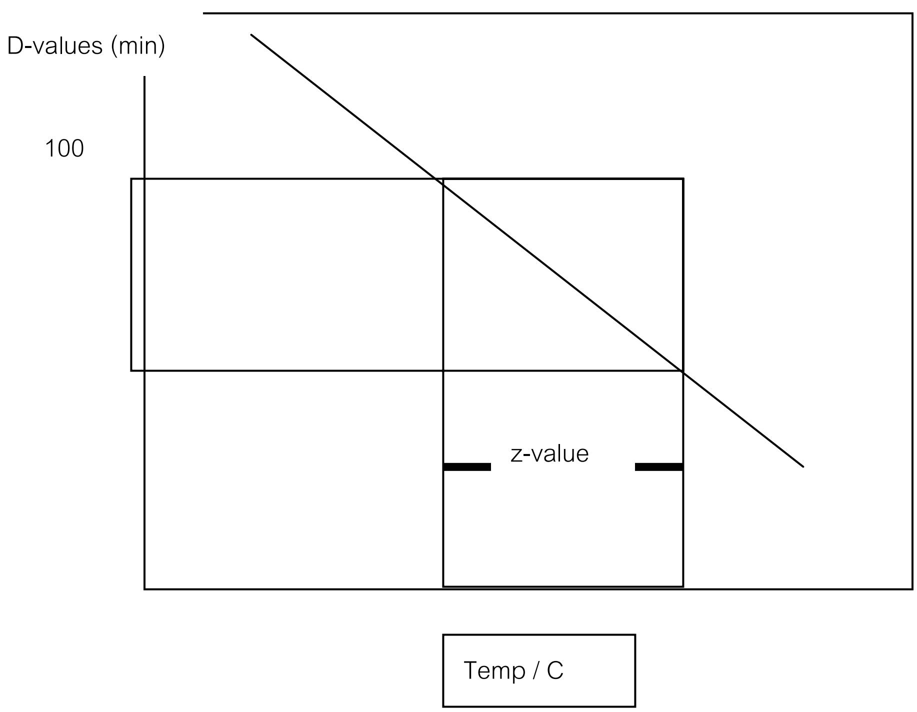
- Mild heat treatment (below 100C)
- Extend shelf life to days (milk) to months (fruit juice)
- Inactivation of enzymes and destruction of heat sensitive bacteria (non-spore formers), yeast, moulds
- Minimal changes in sensory and nutritional qualities
- Temperature and time used in the process is determined by pH of the food
- Low acid food (pH>4.5), destruction pathogenic bacteria
- Acid food (pH<4.5), destruction spoilage microorganisms and enzymes
- Milk (low acid foods) 63C for 30 min or 71.5C for 15sec
- Equipment: Heat Exchanger (Plate/Tube)
- In-package pasteurization
- Pre-heating/Pasteurizing/ Cooling
- Effect on Foods
- Enzymatic browning in fruit juice: promoted by the presence of oxygen. Therefore fruit juices are deaerated before pasteurization
- No effect on color of milk, other pigments in meat and vegetables
- Whiteness in pasteurized milk is due to homogenization
- Loss of volatile compounds
- Losses of vitamin C and carotene can be minimized by deaeration

Sterilization and Canning

- Destroy all microorganisms and enzymes
- More than 6 months
- Changes in sensory and nutritional values
- Canning, UHT (Ultrahigh Temperature)
- In-container sterilization (temp/time)

- Heat resistance microorganisms depend on
    1. Heating condition
    2. pH of Food
    3. Size of container
    4. Physical state of food
- Heat resistance of Microorganisms

D-value (Decimal Reduction Time): Time needed to destroy 90% of the microorganisms
D-value at different temps can be plotted as TDT curve



z-value: number of Celsius required to obtain 10-fold change in decimal reduction time

- Low acid food (pH>4.5) *Clostridium botulimun* is the most dangerous
- Some heat resistance microorganisms

Bacillus stearothermophilus
Cl. thermosaccharolyticum
Cl. botulinum

D_{121} value : 4 min
(*Bacillus stearothermophilus)* It takes 4 mins at 121C to reduce the number of *Bacillus stearothermophilus* by 90%

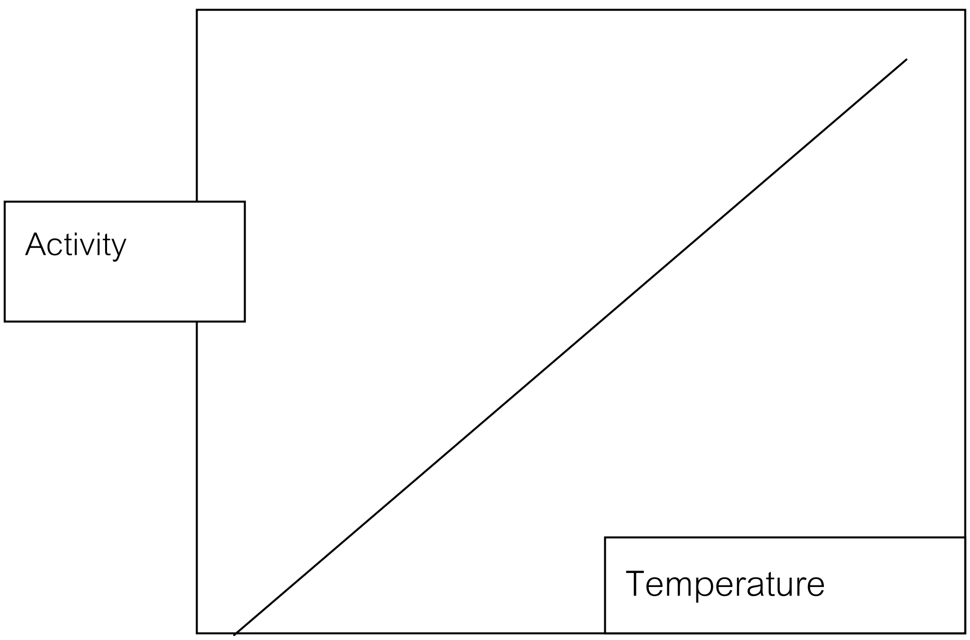
1. Fellows, P. 1990. Food processing technology: principles and practices. Ellis Horwood, New York.
2. Kulshrestha, S. K. 1994. Food preservation. Vikas Publishing House PVT, New Delhi.

CHILLING

- -1 to 8°C
- Reduce Enzyme and microbial activities
- Proper temperatures

-1 to +1°C (fresh meat,fish)
0 to +5°C (milk, cream, yogurt, baked goods, pizzas, sandwiches)
0 to +8°C (fully cooked meat, margarine, fruits, cheese)

- Not all foods can be chilled
- Some fruits suffer from chilling injury at 3-10 °C
- Changes in atmospheric composition can increase the shelf life of chilled foods
- Modified Atmosphere Storage (MAS)
- Modified Atmosphere Package (MAP)



- Shelf life of chilled food

Microbial load
Condition of foods
Temperature distribution
Relative humidity

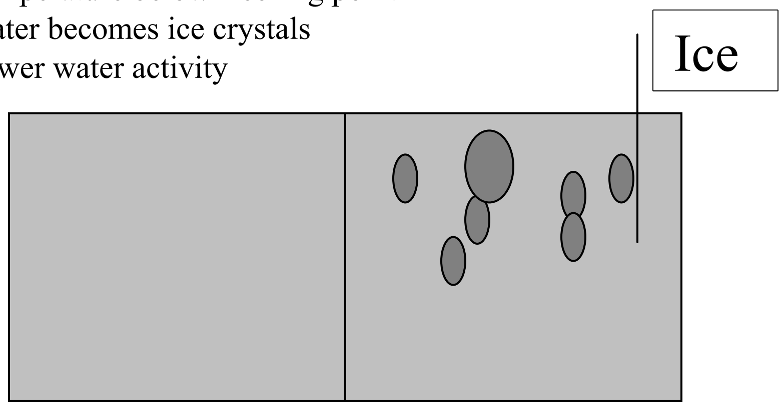
- Chilling Injury: Internal and external browning, failure to ripen and skin blemishes

Apples (less than 2°C), Avocados (less than 4°C), Bananas (less than 12°C), lemons (less than 14°C)

- Effect on foods: little/ no reduction in nutritional properties

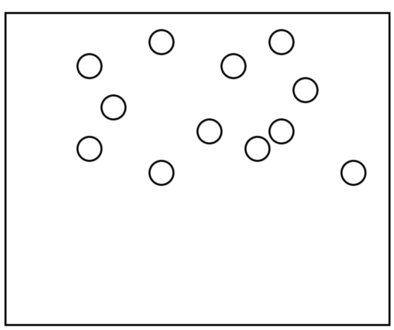
FREEZING

- Temperature below freezing point
- Water becomes ice crystals
- Lower water activity



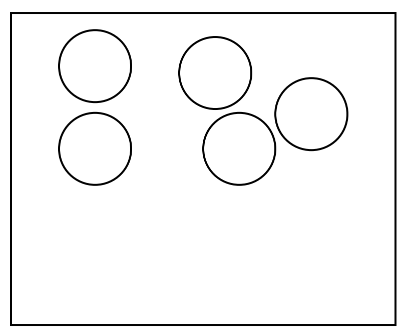
- Changes in pH, viscosity and redox potential of the unfrozen liquor
- Berries, peas, green beans, beef, lamp, poultry, pies, pizzas, ice cream
- Freezing point of a food depends on its composition
- Nucleus of water ➔ crystals

Fast Freezing



Small/many

Slow Freezing



Large/few

<u>Effect on foods</u>

- Large crystals destroy tissues of plant and animal foods
- -4°C to -10°C is more lethal to microorganisms than -15°C to -30°C storage
- Resistance depends on type of microorganisms
- Vegetative cells of yeast and mould and gram negative bacteria is less resistant that gram positive (*Streptococcus aureus* and Enterococci and spores.
- Blanching before freezing helps to reduce microorganisms and inactivate enzymes

- Volume of ice is 9% greater than water
- Recrystallization: changes in size, shape or orientation of crystals)
- Fluctuation of temperature ➔ recrystallization ➔ Quality loss

Dehydration (การทำแห้ง)

- The oldest method of preservation
- By observations the sun drying of nut / grains
- Use of other energy sources to speed up the removal of water
- A_w is reduced, retarding microbial growth
- Microorganisms need free water to grow and multiply
- Sugar, coffee, milk, flour, nuts, breakfast cereal, tea and spices
- Equipment

Tray drier
Belt drier
Fluidized bed drier
Spray drier (milk powder)
Drum drier or roller drier

- Effect on Foods

Color: carotenoid and chlorophyll by heat and oxidation during drying
Nutritive value: Thiamin, Vc
Texture: rupture, distort, and shrink
Aroma: volatile compounds
Partial Protein denaturation in milk powder: reduction in milk solubility and loss of clotting ability and reduced biological value (8-30%)

Fermentation

Controlled action of selected microorganisms

- Alter the texture of foods and preserve foods by production of acids or alcohol.
- Additional flavors and aromas
- Homofermentative microorganisms
- Heterofermentative microorganisms

Lactic acid fermentations:
Produce lactic acid and others and tolerant to the acid (1% up to 3%)
Streptococcus and *Pediococcus* species: homolactic

Leuconostoc: heterolactic
Lactobacillus: depends

Meat: *Pediococcus cerevisiae*
Lactobacillus plantarum

Lactobacillus curvatus
Milk: *Streptococcus thermophilus*
Lb. Bulgaricus
Cheese: *Str. cremoris*
Str. lactis
Lactobacilli
Vegetables: *Lb. mesenteroides*
Lb. brevis
Lb. plantarum

Alcoholic and Mixed alcohol-acid fermentation:

Vinegar: *Acetobacter aceti*
Tempeh: *Rhizopus oligosporus*
Wine: *Sac. cerevisiae* var. *ellipsoideus*
Sac. carbajali
Sac. oviformis
Sac. chevalieri
Beer: *Sac. cerevisiae, Sac. carlesbergensis*

Salting and Sugaring:

- Reduce Aw in foods
- Salting is to selectively allow some microorganisms that are tolerant to salt to grow and ferment (*Bacillus pumilus* and *Bacillus licheniformis*)
- 3-12 months
- Fish: salt 3:1 or 5:1

- Sugaring in to reduce Aw only, does not ferment
- Sweetened milk, sweetened fruits

Chemical additives

Ref: Nickerson, J. T., and A. J. Sinskey. 1972. Microbiology of foods and food processing. American Elsevier Publishing, New York.

- Non or little nutritive substances

Addition of acids: combined treatment with other methods
- Yeast/Mould can grow better at low pH
- Acetic acid (as vinegar) in Pickle and sauerkraut (+salt), ketchup (+heat)
- GRAS: generally recognized as safe, some still have limitation on uses
- Propionic acids: mould and bacteria inhibitor. Up to 0.32% in flour
- Caprylic acid (C8): mould inhibitor in cheese
- Mechanisms: not known

- Speculated: destroy cell membranes and inhibit some enzyme systems
- Benzoates and parabenzoates: upto 0.1% in the U.S.
- Combine with other treatments (Heating/Refrigerating) in fruits, pickled vegetables
- Benzoate: effective at pH 4
- Sodium benzoate is soluble in water

Oxidizing Agents:

- Calcium hypochlorite and chloramines T release free chlorine which inhibits microorganisms
- Hydrogen peroxide: used in milk/ cheese. Followed by catalase to eliminate residual hydrogen peroxide

Other compounds

Sulfur Dioxide: prevent browning and inhibit yeast and bacteria

For example: add sulfur dioxide 350-600 ppm with sugar to preserve fruits

IN wine and Vinegar making, used as equipment sanitizer.

At 50-75 ppm, added to wine in bulk storage to kill wild yeast.

Mechanism: reacts with carbonyl of carbohydrates, thus preventing their utilization of energy source or reacts with some essential enzymes

Sorbic acid: inhibit yeast and mould and catalase positive bacteria, best at pH < 5

Does not have an effect on Clostridia (catalase negative)

Sodium Nitrite: $NaNO_2$ (200ppm) added to cured meat to fix color.

Inhibit bacteria when pH of foods < 6.4

IRRADIATION

- Since 1950's; beta and gamma rays
- Beta rays are generated in an accelerator while gamma rays are emitted by a radioactive source, such as Cobalt 60.
- Cold sterilization: 2-7 Megarads
- 6000-9000 enough to inhibit sprouting of potatoes
- 40,000-60,000 used in an insect disinfection of white flour

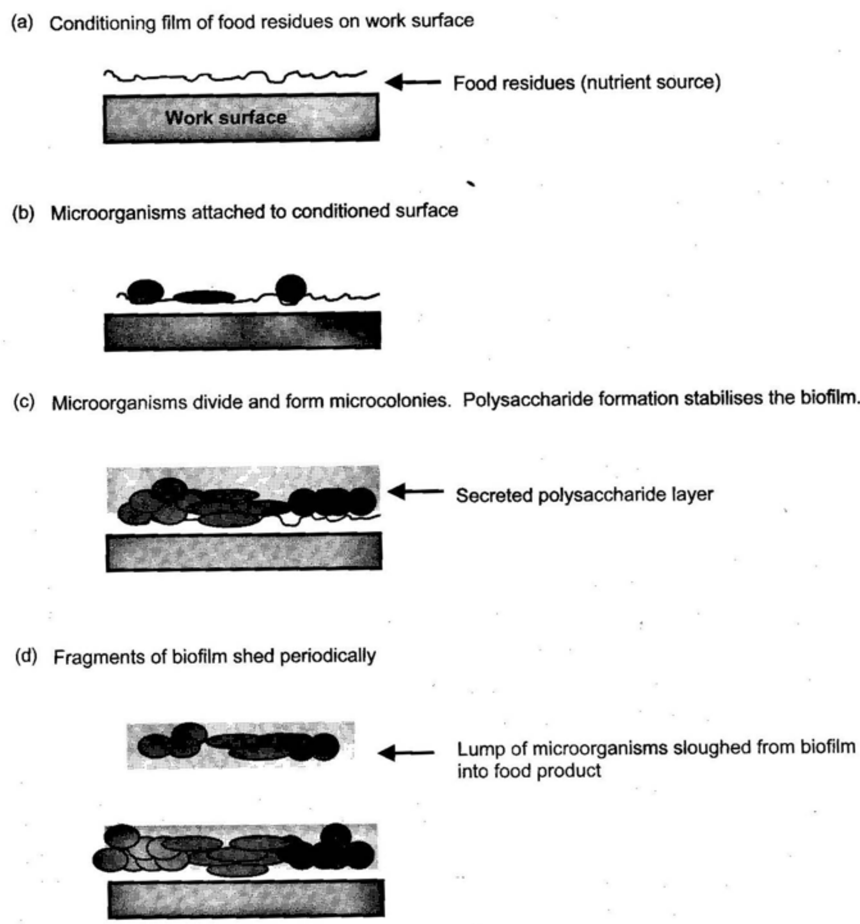
QUESTIONS:

1. Give two examples of extrinsic factors that affect microbial growth
2. Give two examples of intrinsic factors that affect microbial growth
3. What is considered food spoilage
4. Name one microorganism that is psychrotropic
5. Name one microorganism that cab produce spores
6. What is the desirable color pigment in meat
7. What compounds give stale and rancid flavors in fat/oil?
8. How can we prevent enzymatic browning reaction
9. What are the differences between pasteurization and sterilization?
10. What is the pH of low acid foods?
11. What is D-value?
12. What is z-value?
13. What does it mean by $D_{121°C}$ value of 4 min?
14. Name one bacteria that is heat resistant?
15. Why quick-freezing results in small and many ice crystal? *Faster heat removal, thus nucleus of water can be formed easily everywhere*
16. What are the effects of recrystallization on foods?
17. Name two preservation methods that can prevent food spoilage by lowing A_w?
18. What microorganisms used in yogurt fermentation?
19. What microorganisms used in beer and wine fermentation?
20. What does GRAS stand for?

Food Microbiology Lecture 9
Biofilm Formation

- **Biofilms:** Layers of microorganisms growing on surfaces, usually in aquatic environments, as microbial community. They usually produce slime layer (variety of polysaccharides).
- A.S.K.A. Biofouling, Microbial mats
- Embedded cells, Biofilm cells or sessile cells VS
- Planktonic cells or suspended cells
- Biofilm formation:

1. Nutrients absorbed to the surface to form conditioning film. This affects physicochemical properties of the surface, i.e. surface free energy, hydrophobicity and electrostatics charges.
2. Microorganisms attach to the conditioned surface. Initially reversible (van der Walls attraction forces, electrostatic forces or hydrophobic interactions). Finally irreversible (dipole-dipole interactions, hydrogen or ionic bonding). Appendages flagella, fimbriae and exopolysaccharide. Exopolysaccharide is important in cell-cell adhesion and protects cells from dehydration.
3. Grow and produce more polymer. More stable colony
4. Detachment (sloughing). Contaminate the food or initiate a new biofilm.



<u>Biofilm Formation</u>

Sites that attract biofilm formation:
Pipelines, joints, gaskets. Surface cracks etc.

<u>Biofilm removal:</u>

1. Use detergent to remove polysaccharides and food residuals
2. Disinfectant kills microorganisms
3. Rinse to remove the dead and injured cells

<u>Advantages of sessile growth mode</u>

1) Protection from antimicrobial agents;
2) Increased availability of nutrients for growth
3) Increased binding of water molecules
4) Reducing the possibility of desiccation;
5) Establishment of complex consortia, which enhances advantages 1-3
5) Proximity to progeny and other bacteria, facilitating plasmid transfer